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Novel, closely related, white spot syndrome virus (WSSV) genotypes from Madagascar, Mozambique and the Kingdom of Saudi Arabia

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ABSTRACT: White spot syndrome virus (WSSV) is highly pathogenic to penaeid shrimp and has caused significant economic losses in the aquaculture industry around the world. During 2010 to 2012, WSSV caused severe mortalities in cultured penaeid shrimp in Saudi Arabia, Mozambique and Madagascar. To investigate the origins of these WSSV, we performed genotyping analyses at 5 loci: the 3 open reading frames (ORFs) 125, 94 and 75, each containing a variable number of tandem repeats (VNTR), and deletions in the 2 variable regions, VR14/15 and VR23/24. We categorized the WSSV genotype as $\{N_{125}, N_{94}, N_{75}, \Delta X_{14/15}, \Delta X_{23/24}\}$ where N is the number of repeat units in a specific ORF and ΔX is the length (base pair) of deletion within the variable region. We detected 4 WSSV genotypes, which were characterized by a full-length deletion in ORF94/95, a relatively small ORF75 and one specific deletion length in each variable region. There are 2 closely related genotypes in these 3 countries: $\{6_{125}, del_{94}, 3_{75}, \Delta 5950_{14/15}, \Delta 10971_{23/24}\}$ and $\{7_{125}, del_{94}, 3_{75}, \Delta 5950_{14/15}, \Delta 10971_{23/24}\}$, where del is the full-length ORF deletion. In Saudi Arabia, 2 other related types of WSSV were also found: $\{6_{125}, 7_{94}, 3_{75}, \Delta 5950_{14/15}, \Delta 10971_{23/24}\}$ and $\{8_{125}, 13_{94}, 3_{75}, \Delta 5950_{14/15}, \Delta 10971_{23/24}\}$. The identical patterns of 3 loci in these 4 types indicate that they have a common lineage, and this suggests that the WSSV epidemics in these 3 countries were from a common source, possibly the environment.

KEY WORDS: WSSV genotyping · Variable number of tandem repeats · VNTR analysis · Variable-length deletion · Africa · Saudi Arabia

INTRODUCTION

Viral diseases have caused significant economic losses for the shrimp farming industry throughout the world. Among these, white spot syndrome virus (WSSV) caused severe mortalities in many species of penaeid shrimp and other aquatic crustaceans (Lightner 1996, Lo et al. 1996), and infections can result in 95 to 100% mortalities in shrimp ponds. Economic losses in production and trade approach US$10 billion (Stentiford et al. 2009, Lightner 2011).

WSSV is a large (70–150 × 275–380 nm), enveloped, double-stranded DNA virus with a genome size over 300 kb (Van Hulten et al. 2001, Yang et al. 2001). The viral genome has 181 non-overlapping open reading frames (ORFs). Only about 20% of the genes are known to encode structural proteins and those involved in DNA replication or in modifying other proteins. WSSV was first reported around 1991 to 1992 in SE Asia (Huang et al. 1994, Inouye et al. 1994). The virus spread rapidly, and by the late 90s, it had severely impacted most shrimp farming regions. However, shrimp farms in the Indian Ocean remained free of WSSV until recently, when the virus was found in Mozambique (OIE 2011) and Madagascar (OIE 2012a). Before these reports, WSSV was detected in shrimp farms in Saudi Arabia and caused >95% mortalities (Tang et al. 2012).

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To investigate the origins of WSSV emergence within this Indian Ocean–Red Sea region, we applied variable number of tandem repeats (VNTR) analysis and determined the deletion lengths in specific regions within the viral genome to genotype WSSV isolates collected from this region. The analyses of VNTR and variable-length deletions have been used in several epidemiological studies of WSSV (Wongteerasupaya et al. 2003, Dieu et al. 2004, Marks et al. 2004, Pradeep et al. 2008, Muller et al. 2010, Hoa et al. 2012). It has been suggested that deletion lengths in the variable regions VR14/15 and VR23/24 are associated with geographic locations (Dieu et al. 2010), and the VNTR analyses in ORFs 125, 94 and 75 are used for epidemiological comparisons among farms or ponds. In this study, we applied the WSSV genotyping analyses within these 5 loci to compare 13 WSSV representative isolates collected in Madagascar, Mozambique, and Saudi Arabia.

MATERIALS AND METHODS

Shrimp samples

Samples of *Penaeus monodon* and *P. indicus* (taxonomy according to Holthuis 1980) were collected from 2 farms on the west coast of Madagascar (labeled as Farms MG-A and MG-B in Fig. 1), 1 farm in Mozambique (MZ in Fig. 1), and 4 farms along the Red Sea from central to southern Saudi Arabia (SA-A, -B, -C, -D in Fig. 1) between 2010 and 2012 (Table 1). Each sample consisted of whole shrimp or pleopods sampled from 1 to 5 individuals. These were preserved in

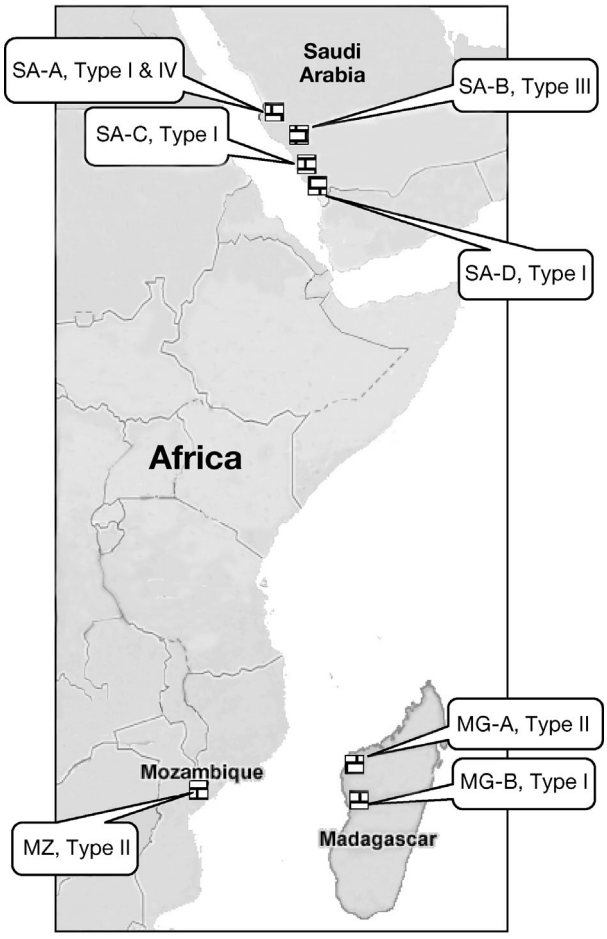

Fig. 1. Locations of the white spot syndrome virus (WSSV)-affected shrimp farms in Madagascar (2 farms: MG-A and MG-B), Mozambique (1 farm: MZ), and Saudi Arabia (4 farms: SA-A to SA-D). The WSSV genotypes detected are indicated

Table 1. Number of repeat units (RU) found within open reading frames (ORFs) 125, 94 and 75, and the length of deletions within variable regions VR14/15 (compared with WSSV TH-96-II; AY753327) and VR23/24 (compared with Taiwan WSSV; AF440570) of WSSV isolated from Madagascar (MG), Mozambique (MZ) and Saudi Arabian (SA) farms (farm sites indicated in Fig. 1). *P.*: *Penaeus*; del: full-length ORF deletion

Farm site	Host species	No. of RU			Deletion size (bp)		Isolate no.	Sampling date
		ORF125	ORF94	ORF75	VR14/15	VR23/24		
MG-A	Wild *P. indicus*	6	del	3	5950	10971	12-354A1	Sep 2012
MG-A	*P. monodon*	6	del	3	5950	10971	12-354A2	Sep 2012
MG-B	*P. monodon*	7	del	3	5950	10971	12-171B	Apr 2012
MG-B	*P. monodon*	7	del	3	5950	10971	12-213F	May 2012
MZ	*P. monodon*	6	del	3	5950	10971	11-312	Sep 2011
SA-A	*P. indicus*	6	7	3	5950	10971	10-143	Apr 2010
SA-A	*P. indicus*	7	del	3	5950	10971	12-380D	Oct 2012
SA-A	*P. indicus*	7	del	3	5950	10971	12-404	Dec 2012
SA-B	*P. indicus*	8	13	3	5950	10971	11-065/6	Feb 2011
SA-B	*P. indicus*	8	13	3	5950	10971	11-102/1	Mar 2011
SA-C	*P. indicus*	7	del	3	5950	10971	11-041/20	Jan 2011
SA-C	*P. indicus*	7	del	3	5950	10971	11-208/3	May 2011
SA-D	*P. indicus*	7	del	3	5950	10971	11-394/B	Nov 2011

95% ethanol and sent to the Aquaculture Pathology Laboratory at the University of Arizona, Tucson USA, for PCR analysis for the presence of WSSV. The presence of the virus was determined by PCR using a World Organisation for Animal Health (OIE) recommended method (procedure is described below).

DNA extraction, WSSV PCR analysis and genotyping

Total DNA was extracted with a Maxwell-16 Cell LEV DNA purification kit (Promega). For WSSV detection, a nested PCR was performed with PuReTaq™ Ready-To-Go™ PCR beads (GE Healthcare). Each reaction (final volume: 25 µl) contained 1 µl of extracted DNA (at concentrations of 100 to 300 ng μl^{-1}), 2.5 units of PuReTaq™ DNA polymerase, 10 mM Tris-HCl, pH 9.0, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM each dNTP, and 0.2 µM WSSV primers (first step primers: 146F1 and 146R1; second step primers: 146F2 and 146R2; OIE 2012b). Amplifications (both first and second step) were carried out as initial denaturation at 94°C for 2 min, followed by 30 cycles of 94°C for 30 s, 62°C for 30 s, and 72°C for 30 s; and a final extension at 72°C for 2 min; Following this, an aliquot of the PCR mixture was analyzed in a 1.2% agarose gel containing ethidium bromide and then photographed with an AlphaImager gel documentation system (Alpha Innotech).

For VNTR analysis within the 3 ORF regions (ORFs 125, 94 and 75) and determination of the length of deletions in the 2 variable regions (VR14/15 and VR23/24), PCR was performed using the Ready-To-Go™ PCR beads with specific primers (Table 2). PCR results were visualized as described above. The PCR products were purified with the QIAquick PCR purification kit (Qiagen), and DNA sequencing was performed by the Genomic Analysis and Technology Core facility at University of Arizona by a 3730 DNA Analyzer (Applied Biosystems).

From the nucleotide sequence, the number of repeat units (RU) within the ORFs was determined with the Tandem Repeats Finder program (Benson 1999). The consensus sequence of the RU in each ORF is as follows: ORF125 (69 bp) – AG/TA ACA AGC AGG AAG AAG ACG CGA GGA TCA AGC GTG CAG TCG ACA TGG CTG TTG CAG CCA TCA ACG AAA; ORF94 (54 bp) – CGC AAA AAG CGT GCC GCA CCT CCA CCT GAG GAT GAA GAA GAG GAT GAT TTC TAC; ORF75 (45 bp) – GAA GCA GCT CCC CCA CTT AAA GGT GCA CTT GGA CGT AAG AGG CGC; ORF75 (102 bp) – GAA GCA GCT

Table 2. PCR primers and cycling conditions used for genotyping within open reading frames (ORFs) 125, 94 and 75, and variable regions VR14/15 and VR23/24

Primer	Sequence (5' to 3')	Cycling conditions			Reference
		Initial	Amplification	Final	
ORF125-flank-F	CGA AAT CTT GAT ATG TTG TGC	94°C for 3 min	35 cycles: 94°C for 30 s, 55°C for 30 s, 72°C for 1 min	72°C for 7 min	Dieu et al. (2004)
ORF125-flank-R	CCA TAT CCA TTG CCC TTC TC				
ORF94-F	TCT ACT CGA GGA GGT GAC GAC	94°C for 3 min	35 cycles: 94°C for 30 s, 55°C for 30 s, 72°C for 1 min	72°C for 7 min	Wongteerasupaya et al. (2003)
ORF94-R	ACA GGT GTG TAC ACA TTT CAT G				
ORF73-F	CTT TCA CCG CTC TCA CCA AC	94°C for 3 min	35 cycles: 94°C for 30 s, 55°C for 30 s, 72°C for 2 min	72°C for 7 min	Present study
ORF77-R	GGG TTC ACC AGA GAG ACA GG				
VR14/15-complete-(F)	AAT ATG GAA CGA CGG GTG	94°C for 3 min	35 cycles: 94°C for 30 s, 50°C for 30 s, 72°C for 1 min	72°C for 7 min	Marks et al. (2005)
VR14/15-complete-(R)	GAC CAG CGC CTC TTC AG				
VR23/24-south-(F)	GTA GTG CAT GTT TCT CTA AC	94°C for 3 min	35 cycles: 94°C for 30 s, 45°C for 30 s, 72°C for 2 min	72°C for 7 min	Dieu et al. (2010)
VR23/24-south-(R)	GTA AGT TTA TTG CTG AGA AG				

CCT CCA CTT AAA GGT GCG CTT GGA CGT AAG AGG CGC GAA GCA GAA TCC TTG GAG GAA GAA CTT GTG TCT GCT GAA GAA GAA CGT GAA AAG CGC. The variable-length deletions within VR14/15 and VR23/24 were compared with the reference strains of TH-96-II (Genbank no. AY753327) and Taiwan (AF440570), respectively (Dieu et al. 2004, 2010, Zwart et al. 2010). The WSSV genotype was tentatively categorized as {N_{125}, N_{94}, N_{75}, $\Delta X_{14/15}$, $\Delta X_{23/24}$} where N is the number of repeat units in a specific ORF, and the subscript indicates the ORF, and ΔX is the length (base pair) of deletion within the variable region (VR14/15, VR23/24).

RESULTS

Detection of WSSV in Saudi Arabia and Africa during 2010 to 2012

From 2010 to 2012, samples from shrimp farms in Saudi Arabia, Mozambique, and Madagascar (Fig. 1) were collected for determining the cause of severe mortality; the results showed that WSSV was detected by PCR. The WSSV infection was confirmed by an *in situ* hybridization (data not shown). From the WSSV-positive DNA samples, we performed genotyping analyses in 5 commonly used loci: ORFs 125, 94, 75, and variable regions VR14/15 and VR23/24.

VNTR analysis in ORF125

The PCR targeting ORF125 generated 3 different sizes of amplicons: 652, 722 and 792 bp. These amplified fragments were found to contain 6, 7 and 8 RU (Table 1), respectively. The WSSV from the MG-A farm contained 6 RU in both cultured *Penaeus monodon* and wild *P. indicus* (Table 1). The WSSV from the MG-B farm consisted of 7 RU. The Mozambique WSSV contained 6 RU identical to those from MG-A. From the 4 farms in Saudi Arabia, WSSV was detected with 6, 7 or 8 RU; the 2 most recent samples (Table 1, isolate no. 12-380D and 12-404) were detected with 7 RU. The 8 RU was only detected from the SA-B farm.

VNTR analysis in ORF94

Within ORF94, we carried out the PCR with the primers ORF94-F and ORF94-R. None of the Madagascar and Mozambique WSSV isolates were amplified (Table 1), nor were the 5 Saudi isolates. These 10 isolates generated a 348 bp DNA fragment when amplified with primers ORF93-F1 and ORF96-R1 (data not shown; primers sequences are described in Tang et al. 2012), indicating that only one length of deletion was detected; this deleted fragment represents the full-length deletions in both ORF94 and ORF95 (illustrated in Tang et al. 2012). In 3 representative Saudi isolates collected in 2010 and 2011, 7 or 13 RU were detected.

VNTR analysis in ORF75

To determine the number of RU in ORF75, we first performed PCR with primers ORF75-flank-F and ORF75-flank-R (Dieu et al. 2004, Pradeep et al. 2008). No PCR products were detected. Thus, we selected a new pair of primers, ORF73-F and ORF77-R, that targeted ORF73 and ORF77, respectively. These primers amplified a 1739 bp fragment in all 13 samples. The nucleotide sequence of this fragment was compared with 4 full-length WSSV ORF75 from Taiwan (GenBank no. AF440570, containing 21 RU; Marks et al. 2004), China (AF332093, 15 RU), Thailand (AF369029, 12 RU) and Korea (JX515788). There was a 1297 bp deletion in the WSSV from this region (Madagascar, Mozambique, and Saudi Arabia) when compared with the Taiwan isolate, which has the largest ORF75; only a 574 bp deletion was found for ORF75. This large deletion encompasses the target site for ORF75-flank-F and thus had led to no amplification. This 574 bp ORF75 contains 3 RU: one 102 bp RU and two 45 bp RU (Fig. 2). We also found that the Korea WSSV has a near 1 kb deletion and contains only 4 RU, which is relatively small compared with those found in the 3 other Asia WSSV.

Deletion in VR14/15

To determine the length of deletion in VR14/15, we performed PCR with its specific primers. All 13 isolates generated a PCR fragment of 1851 bp; this corresponds to a 5950 bp deletion when compared with an ancestral strain, TH-96-II (AY753327), which is estimated to ~312 kb (Marks et al. 2005) (Fig. 3A, Table 1). This deletion length is not specific to WSSV found in this region; the same deletion was found in an Indian WSSV (IN-05, EU327501) and WSSV from southern Vietnam (Hoa et al. 2012). A slightly smaller deletion (5892 bp) was found in 4 other WSSV: Indian isolate IN-07 (EF468499) and 3 Mexico isolates

(HQ257380, HQ257383, HQ257381). The sizes of deletion range from 5132 to 5721 bp in the 4 Asia isolates (Taiwan, China, Thailand and Korea).

Deletion in VR23/24

PCR targeting VR23/24 generated amplified products of 1264 bp in all 13 samples. This corresponds to a 10 971 bp deletion when compared with the genome of Taiwan WSSV (Fig. 3B, Table 1). This deletion size is large compared with others, and the same length of deletion was also found in 2 Indian WSSVs, IN-05-I and -II (EU327499, EU327500). Overall, the length of deletion in this region is more variable, ranging from 1 to 13 kb. The Thailand WSSV (AF369029) has been shown to have the largest deletion (13 210 bp; Pradeep et al. 2008), the Korea WSSV has a medium deletion (5654 bp) and China WSSV has a smaller deletion (1169 bp).

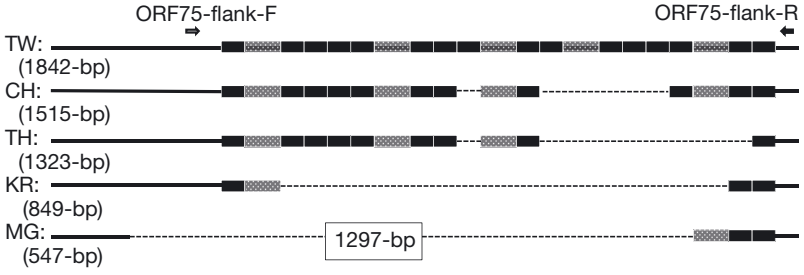

Fig. 2. Schematic alignment of white spot syndrome virus (WSSV) open reading frame ORF75 (full-length) from Taiwan (TW, 1842 bp, used as reference genome), China (CH, 1515 bp), Thailand (TH, 1323 bp), Korea (KR, 849 bp) and Madagascar (MG, 547 bp). ▬: 45 bp repeat unit; ▩: 102 bp repeat unit; ➡ and ⬅: target sites for primers ORF75-flank-F and ORF75-flank-R, respectively. Solid lines indicate the sequences that are maintained in the genome; dashed lines indicate the deleted sequences

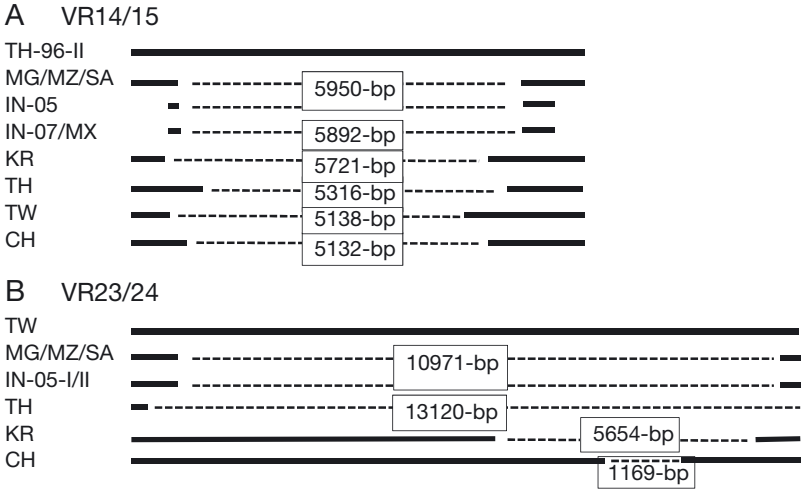

Fig. 3. Schematic alignment within (A) variable region VR14/15 — the Thailand (TH-96-II, AY864666) isolate is shown as the reference genome — and (B) VR23/24, the Taiwan (TW, AF440570) isolate is shown as the reference genome. Solid lines indicate the sequences that are maintained in the genome; dashed lines indicate the deleted sequences. The length of deletion is indicated within the text box. MG: Madagascar; MZ: Mozambique; SA: Saudi Arabia; CH: China; KR: Korea; IN-05, IN-05-I and -II: Indian isolates; IN-07/MX: Indian isolate IN-07 and 3 Mexico isolates

Four WSSV genotypes

From these 13 isolates, 4 genotypes were found, designated as Type I ({7_{125}, del_{94}, 3_{75}, $\Delta5950_{14/15}$, $\Delta10971_{23/24}$}), Type II ({6_{125}, del_{94}, 3_{75}, $\Delta5950_{14/15}$, $\Delta10971_{23/24}$}), Type III ({8_{125}, 13_{94}, 3_{75}, $\Delta5950_{14/15}$, $\Delta10971_{23/24}$}) and Type IV ({6_{125}, 7_{94}, 3_{75}, $\Delta5950_{14/15}$, $\Delta10971_{23/24}$}). Type I was the most prevalent type and was found in 1 Madagascar farm (MG-B) and 3 Saudi farms (SA-A, -C and -D) (Fig. 1); Type II was found in the other Madagascar farm (MG-A) and the Mozambique farm (MZ). Type III was only found in 1 Saudi farm (SA-B). Type IV was isolated from a Saudi farm (SA-A) in 2010, but this type was not detected in 2011 or 2012.

These 4 types seem to be closely related. All 13 isolates have the identical sequence in ORF75, VR14/15 and VR23/24: {3_{75}, $\Delta5950_{14/15}$, $\Delta10971_{23/24}$}. Among these, 10 isolates also had an identical deletion pattern in ORF94/95. The deletions in ORF94/95 and ORF75 were not seen in isolates elsewhere. The ORF125 is more variable — 3 types of RU were observed.

DISCUSSION

Four genotypes of WSSV were recently (2010 to 2012) found in shrimp ponds in Saudi Arabia, Mozambique, and Madagascar. All 4 have identical genomic patterns in 3 of the 5 loci examined, suggesting a common lineage among these isolates. Two of the genotypes (Types I and II) are more closely related, with one repeat unit (69 bp) difference in ORF125. Both types appeared during 2011 to 2012 at the peak of WSSV epidemics in these 3 countries. These 2 genotypes (Types I and II) have full-length deletions in ORF94/95 and a

reduced size ORF75 — features that have not been reported in other regions. Type I was the most widespread, detected in 7 out of the 13 representative isolates from 3 countries. Type II was found in Mozambique and Madagascar but was not in any of the Saudi farms. Type III was only found in one Saudi (SA-B). Type IV was found only in Saudi farm (SA-A) in 2010 but seems to have disappeared, as samples from this farm have been Type I since 2011.

These genotypes have not been found elsewhere and probably evolved in this region. WSSV detected in these 3 countries likely originated from wild populations in the Red Sea and Indian Ocean, based on the fact that these countries have strict regulations for importation of livestock. Also, different species are cultured in Saudi Arabia than in either Mozambique or Madagascar. Saudi farms culture *Penaeus indicus*, while *P. monodon* is cultured in Mozambique and Madagascar. Therefore, clearly WSSV from Mozambique and Madagascar did not come from imported stock from Saudi Arabia. In addition, wild crustaceans were positive for WSSV along the Mozambique and Madagascar coasts, including wild stocks of *P. indicus*.

The WSSV genome evolves through shrinking, and isolates with smaller genomes tend to be more virulent (Marks et al. 2005, Zwart et al. 2010). The evolution of WSSV appears to occur through gradual deletions in variable regions VR14/15 and VR23/24 (Dieu et al. 2004, Marks et al. 2005). The WSSV types from our study have substantially reduced genome sizes compared with presumptive ancestral isolates from Taiwan and Thailand. Because of the reduced genome size, elevated virulence of these new isolates would be suspected, although this remains to be verified through controlled studies.

LITERATURE CITED

Benson G (1999) Tandem repeats finder: a program to analyze DNA sequences. Nucleic Acids Res 27:573–580

Dieu BTM, Marks H, Siebenga JJ, Goldbach RW, Zuidema D, Duong TP, Vlak JM (2004) Molecular epidemiology of white spot syndrome virus within Vietnam. J Gen Virol 85:3607–3618

Dieu BTM, Marks H, Zwart MP, Vlak JM (2010) Evaluation of white spot syndrome virus variable DNA loci as molecular markers of virus spread at intermediate spatiotemporal scales. J Gen Virol 91:1164–1172

Hoa TTT, Zwart MP, Phuong NT, Oanh DTH, de Jong MCM, Vlak JM (2012) Indel-II region deletion sizes in the white spot syndrome virus genome correlate with shrimp disease outbreaks in southern Vietnam. Dis Aquat Org 99:153–162

Holthuis LB (1980) Shrimp and prawn of the world: an annotated catalogue of species of interest to fisheries. FAO species catalog. FAO Fisheries Synopsis 125 (1). FAO, Rome

Huang J, Song XL, Yu J, Yang CH (1994) Baculoviral hypodermal and haematopoietic necrosis pathology of the shrimp explosive epidemic disease. Yellow Sea Fishery Research Institute, Qingdao (Abstract)

Inouye K, Miwa S, Oseko N, Nakano H, Kimura T, Momoyama K, Hiraoka M (1994) Mass mortality of cultured kuruma shrimp *Penaeus japonicus* in Japan in 1993: electron microscopic evidence of the causative virus. Fish Pathol 29:149–158

Lightner DV (1996) A handbook of shrimp pathology and diagnostic procedures for diseases of cultured penaeid shrimp. World Aquaculture Society, Baton Rouge, LA

Lightner DV (2011) Virus diseases of farmed shrimp in the Western Hemisphere (the Americas): a review. J Invertebr Pathol 106:110–130

Lo CF, Ho CH, Peng SE, Chen CH and others (1996) White spot syndrome baculovirus (WSBV) detected in cultured and captured shrimp, crabs and other arthropods. Dis Aquat Org 27:215–225

Marks H, Goldbach RW, Vlak JM, van Hulten MCW (2004) Genetic variation among isolates of white spot syndrome virus. Arch Virol 149:673–697

Marks H, van Duijse JJA, Zuidema D, van Hulten MCW, Vlak JM (2005) Fitness and virulence of an ancestral white spot syndrome virus isolate from shrimp. Virus Res 110:9–20

Muller IC, Andrade TPD, Tang-Nelson KFJ, Margues MRF, Lightner DV (2010) Genotyping of white spot syndrome virus (WSSV) geographical isolates from Brazil and comparison to other isolates from the Americas. Dis Aquat Org 88:91–98

OIE (World Organisation for Animal Health) (2011) Immediate notification, submitted date: 22/09/2011. OIE, Paris

OIE (World Organisation for Animal Health) (2012a) Immediate notification, submitted date: 09/05/2012. OIE, Paris

OIE (World Organisation for Animal Health) (2012b) Manual of diagnostic tests for aquatic animals. OIE, Paris

Pradeep B, Shekar M, Gudkovs N, Karunasagar I, Karunasagar I (2008) Genotyping of white spot syndrome virus prevalence in shrimp farms of India. Dis Aquat Org 78:189–198

Stentiford GD, Bonami JR, Alday-Sanz V (2009) A critical review of susceptibility of crustaceans to Taura syndrome, yellowhead disease and white spot disease and implications of inclusion of these diseases in European legislation. Aquaculture 291:1–17

Tang KFJ, Navarro SA, Pantoja CR, Aranguren FL, Lightner DV (2012) New genotypes of white spot syndrome virus (WSSV) and Taura syndrome virus (TSV) from the Kingdom of Saudi Arabia. Dis Aquat Org 99: 179–185

Van Hulten MCW, Witveldt J, Peters S, Kloosterboer N and others (2001) The white spot syndrome virus DNA genome sequence. Virology 286:7–22

Wongteerasupaya C, Pungchai P, Withyachumnarnkul B, Boonsaeng V, Panyim S, Flegel TW, Walker PJ (2003) High variation in repetitive DNA fragment length for white spot syndrome virus (WSSV) isolates in Thailand. Dis Aquat Org 54:253–257

Yang F, He J, Lin X, Li Q, Pan D, Zhang X, Xu X (2001) Complete genome sequence of the shrimp white spot bacilliform virus. J Virol 75:11811–11820

Zwart MP, Dieu BTM, Hemerik L, Vlak JM (2010) Evolutionary trajectory of white spot syndrome virus (WSSV) genome shrinkage during spread in Asia. PLoS ONE 5: e13400

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